channels (MSCs) in the breast cancer cell line MCF7 with patch clamp methods.
MSCs were present in 132 out of 258 cell-attached membrane patches. MSCs could be activated by negative pressure at the outer side of the membrane in a saturable manner (EP50: 41.2 ± 0.5 mbar (N=13).When K^+ was predominantly present in the extracellular pipette solution, single channel conductance exerted to be 25.6 ± 0.4 pS (N=8). When complete ion selectivity was assessed, conductivity was found to increase in the following order: Li+ < Na^+ < K^+ ≈ Rb+ ≈ Cs+. Furthermore, conductivity was smaller for divalent cations (100 mmole/L compared to 153 mmole/L) with the order: Ca^{2+} < Ba2+ < K + ≈ Rb + ≈ Cs +. We compared the biophysical properties of MSCs with a recently discovered mechanosensitive cation channel, the human Piezo1 protein, heterologously overexpressed in HEK293 cells. Single channel conductances were indistinguishable between MSCs from MCF7 and hPiezo1 in HEK293 cells, both when 100% K + and 50% K + / 50% Na + were used as permeant ions.
MSCs occur in MCF7 breasts cancer cells, providing a sensorium for mechanical stress. Ion selectivity studies show that divalents like Ca^{2+} can permeate to a considerable extend. Moreover, permeation of monovalents is apparently restricted by the size of the ions hydrate shell, as indicated by the smaller conductivities for Na + and Li +, respectively, indicating that the ion permeation mechanism through MSCs may be different to the cation channels crystallized so far.
This research is supported by grants from the Austrian Research Foundation (FWF22974 (WS); KLIF182 (TB)).

2783-Pos Board B475
Diminazene Interaction with ASIC1A Channels
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Diaryldiamidines, a class of DNA minor groove binders used clinically since the 1940s in the treatment of tropical parasitoses such as trypanosomiasis, leishmaniasis, pneumocystis pneumonia and babesiosis, have been recently found to exert specific blocking effects on acid-sensing ion channels (ASIC), raising hopes to develop new small molecule neuroprotective agents for ischemic stroke, which involves acidosis-mediated activation of ASIC1a and heteromeric ASIC1a/2b channels. Therefore we have thoroughly investigated the modes of interaction of diminazene with ASIC1a via whole-cell and single-channel recordings on outside-out patches excised from HEK293 cells of ASIC1a transient currents evoked by brief acidic pulses (pH 6.0). Data idealization and model-dependent fitting were performed with custom hidden Markov model maximum likelihood algorithms. We proved that the 3 subconductance levels are due to partial block by calcium of the permeation pathway, and expanded our previous 8-state linear connectivity gating model (Marin *et al*. 2008 *Channels* 2(6):1-10) including 3 subconductance states for each of the 2 gating modes. Single-channel recordings with diminazene 0.6 or 3 μM indicated a mechanism of open channel block. By exponential fits of the distributions of blocked and unblocked periods for each gating mode we computed binding and unbinding rates and dissociation constants of 0.13 and 0.27 μM for gating modes 2 and 1, respectively. Using atomic resolution models of human ASIC1a in the open conformations corresponding to the 2 gating modes, we found via molecular docking several intra- and intersubunit binding sites for diminazene. One of these sites, assumed to be responsible for the state-dependent block, features protrusion of the drug in the transmembrane domain permeation pathway. As expected, for this blocking site the interaction energy is higher in the symmetrical open conformation of the second gating mode than in the asymmetrical open conformation of the first gating mode.

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Effects of Disease-Associated Mutations on the Conformations of GABA(A) Receptors
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GABA(A) receptors are critical for proper transmission of inhibitory signals in the central nervous system, and are common targets of anesthetic and anxiolytic drugs. Several naturally occurring mutations in such receptors can cause an increased likelihood of seizures. In particular, gamma-K289M on the M2-M3 loop attached to the pore-lining M2 helices is associated with Generalized Epilepsy and Febrile Seizures. At the single channel level, the mutation dramatically reduces current amplitude in some subspecies, and increases deactivation rate in other subspecies. The molecular mechanism through which the mutation causes these effects is unknown. Using a homology model of the GABA(A) receptor based on the recently solved structure for the homologous glutamate-gated chloride channel (GluCl), we ran molecular dynamics simulations of multiple replicas incorporating both the Wild-type and the mutation, at room temperature and at temperatures inducing febrile seizures. We find distinct effects of temperature on the mutant for some conformational variables, including those governing the pore radius. From these results we propose a molecular mechanism for rapid but unstable closure of GABA(A) receptors, which would be likely to cause flickering in single channel recordings.

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Protein Kinase C-Theta Controls the CLC-1 Chloride Channel Function and Skeletal Muscle Phenotype: A Biophysical and Gene Expression Study in Pkc-Theta Null Mice
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In skeletal muscle the resting chloride conductance (gCl), sustained by the ClC-1 chloride channel, controls the sarcolemma electrical stability. Resting gCl is negatively regulated by Protein Kinase C (PKC), since its pharmacological activation closes the ClC-1 channel. A reduced activity of PKC contributes to the low gCl typical of slow-twitch muscles compared to the fast ones. Different PKC isoforms are expressed in skeletal muscle, including the novel isoform PKC-theta, where it mediates various cellular responses. Here we investigated the role of PKC-theta in the regulation of ClC-1 channel activity and expression in extensor digitorum longus (EDL) and in soleus (Sol) muscles, using two models of PKC-theta null mice: a PKC-theta knockout model, in which the PKC-theta gene was inactivated and the mPKC-theta-K/R transgenic model, in which a dominant-negative mutant form of PKC-theta is expressed under a muscle-specific promoter control. Electrophysiological studies showed a gCl increase in Sol and EDL muscle of KO mice with respect to wild-type. Muscle excitability was reduced accordingly. Similar effects were observed in mPKC-theta-K/R mice. By using chelerythrine, a non-specific PKC inhibitor, we demonstrated that other PKC isoforms present in skeletal muscle of PKC-theta null mice are able to further modulate gCl. In parallel, we found that the expression of the ClC-1 channel, evaluated by RT-PCR, was not modified either in EDL or in Sol of PKC-theta-KO mice, demonstrating that PKC-theta does not control the ClC-1 expression but its activity. These results as well as the modification of calcineurin and myocyte-enhancer-factor-2 expression demonstrate the involvement of PKC-theta in the control of muscle phenotype. We conclude that PKC-theta plays a role in regulating ClC-1 chloride channel activity and skeletal muscle function. (ASI-OSMA).

2786-Pos Board B478
Blocking KCa1.1 Channels Inhibits the Pathogenic Features of Fibroblast-Like Synoviocytes and Treats Rat Models of Rheumatoid Arthritis
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Rheumatoid arthritis (RA) is a chronic autoimmune disease that mainly affects synovial joints, resulting in cartilage and bone degradation. Current therapies for RA typically focus on suppressing the immune response, although resident joint cells known as fibroblast-like synoviocytes (FLS) are responsible for many of the pathogenic features of RA. In normal joints, FLS line the capsule and function to maintain the extracellular matrix and to lubricate the joint. However, FLS in RA (RA-FLS) develop an invasive phenotype tightly correlated with joint damage and release proteases and pro-angiogenic and pro-inflammatory growth factors. Due to their pathogenic nature, understanding how to regulate RA-FLS activity could provide the basis for novel therapeutics for RA that would bypass the need for immunosuppressants.
We have found that the KCa1.1 channels are involved in many of the pathogenic features of RA-FLS. KCa1.1 is up-regulated in RA-FLS and localizes on the leading edge of the plasma membrane. Blocking KCa1.1 ex vivo inhibits cellular migration and invasion, along with the production of VEGF, IL-8, and MMP-2. This inhibition in cellular motility agrees with our finding that blocking KCa1.1 inhibits the formation of lamellipodia and interferes with integrin regulation in RA-FLS. These changes in phenotype are likely due to a calcium transient that forms as a result of KCa1.1 block, as invasiveness of KCa1.1-blocked RA-FLS is rescued upon blocking CaV channels on the cell membrane. We have also found that blocking KCa1.1 with the small molecule paxilline significantly decreases clinical signs in two complementary rat models of RA.